

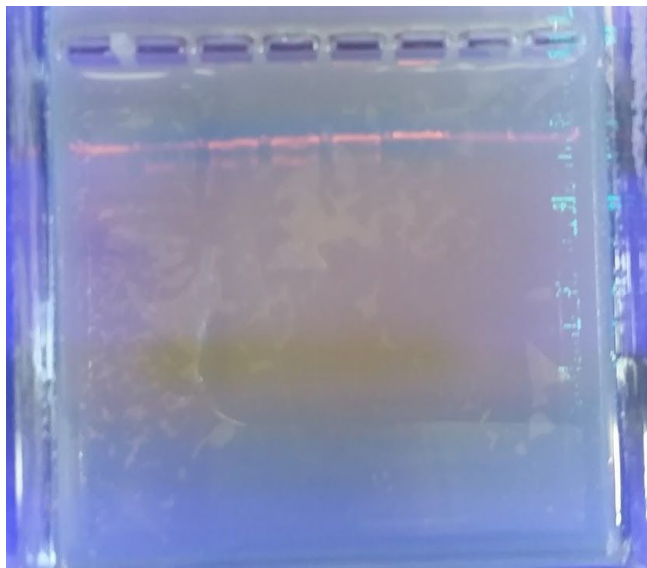
iGEM 2016: Team Pittsburgh  
**Week 18 Lab Notebook**

Monday, September 19

Gel check (Claire)

Lane	1	ladder
	2	PT7-T3
	3	PT3-T3 1
	4	PT3-T3 2
	5	PT3-T3 3
	6	PT3-T3 4

100 V, 35 min



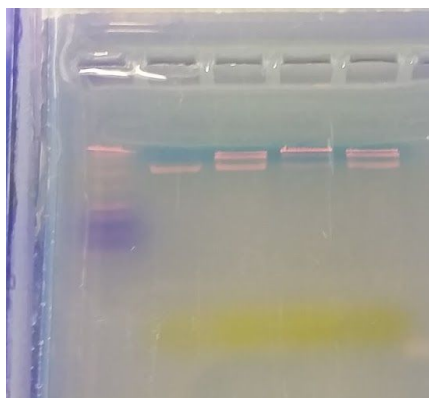
Tuesday, September 20

Gel (Claire)

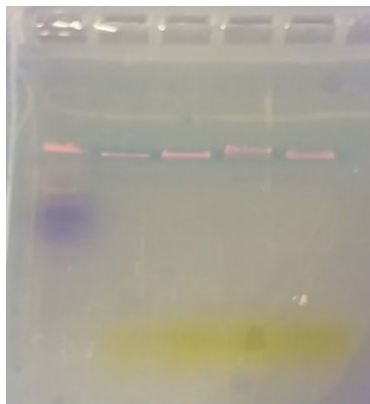
Lane 1:	3 $\mu$ L ladder + 2 $\mu$ L dye
2:	PT7-T3
3:	PT3-T3 1
4:	PT3-T3 2
5:	PT3-T3 3

100 V, 35 min. ladder could separate more.  
Some double bands.

Run 10 more min.  
Ladder only has a few evenly spaced bands  
No double bands ???



35 min



45 min

PT3-GFP Amp (Claire)

5x Phusion buffer	10 $\mu$ L
10mM dNTP	1 $\mu$ L
10 $\mu$ M forward	2.5 $\mu$ L
10 $\mu$ M reverse	2.5
template <del>10 ng</del> = 10 ng	<del>0.42</del> 1.2 $\mu$ L
Phusion DNA pol.	0.5 $\mu$ L
	<del>32.3</del> <del>32.3</del> H <sub>2</sub> O = 50 $\mu$ L
98° 30 s.	
25-35 cycles:	98° 10 s. 64° 30 s.
	72° 2 min
Final	72° 8 (10) min
hold	4-10°

LacZ Mutagenesis (Maya)

Wednesday, September 21

DpnI digest of LacZ (Maddie)

DpnI, Xba, Pst digest of PT3-GFP digest (Maddie)

Gel Checks for both (Maddie)

Friday, September 23

Gel check (Maya)

Lane 1: Ladder

Lane 2: PT3-GFP Control

Lane 3: PT3-T3 amp digested

Lane 4: T7 control

Lane 5: PT7-T3

Lane 6: PT3 Control

Lane 7: PT3-T3 1

Lane 8: PT3-T3 2

*Saturday, September 24*  
Maya/Adam modeling